

Incorporation of strontium, cadmium, and barium in juvenile spot (*Leiostomus xanthurus*) scales reflects water chemistry

Brian K. Wells, Gretchen E. Bath, Simon R. Thorrold, and Cynthia M. Jones

Abstract: We investigated the hypothesis that strontium:calcium (Sr:Ca), cadmium:calcium (Cd:Ca), and barium:calcium (Ba:Ca) composition in scales reflects that of the ambient seawater in which fish were reared under controlled experimental conditions. Juvenile spot (*Leiostomus xanthurus*) were held in replicate tanks containing four different concentrations of Sr, Cd, and Ba maintained at either 20 or 25°C for a total of 42 days. The elemental composition of scales from these fish was analyzed at the termination of the experiment, using isotope dilution inductively coupled plasma mass spectrometry (ICP-MS). Sr, Cd, and Ba levels in the scales, expressed as ratios to Ca, were linearly related to environmental concentrations, indicating that the elements were incorporated in constant proportions to levels in the ambient water. Temperature had no measurable effect on the uptake of Sr, Cd, or Ba into the scales. Finally, Sr:Ca and Ba:Ca ratios in scales were highly correlated with levels in the otoliths from the same treatment. In all, Sr:Ca, Cd:Ca, and Ba:Ca signatures in scales appear to be representative of the ambient environment and, therefore, may be useful for quantifying life-history characteristics of individual fish.

Résumé : Nous avons examiné l'hypothèse selon laquelle la composition des écailles en strontium:calcium (Sr:Ca), cadmium:calcium (Cd:Ca) et baryum:calcium (Ba:Ca) refléterait celle de l'eau de mer ambiante dans laquelle des poissons sont élevés dans des conditions expérimentalement contrôlées. Des juvéniles de tambour croca (*Leiostomus xanthurus*) ont été gardés dans des bassins semblables contenant quatre concentrations différentes de Sr, Cd et Ba et maintenus soit à 20°C soit à 25°C, pendant 42 jours au total. La composition élémentaire des écailles de ces poissons a ensuite été analysée à l'aide de la spectrométrie de masse par dilution isotopique avec plasma inductif, une fois l'expérience terminée. Les concentrations de Sr, Cd et Ba dans les écailles, exprimées par leur rapport avec le calcium, étaient en relation linéaire avec la concentration dans l'environnement, ce qui indique que les éléments se sont incorporés en proportions constantes par rapport à celles de l'eau ambiante. La température n'avait aucun effet mesurable sur l'absorption de Sr, Cd ou Ba dans les écailles. Enfin, les rapports Sr:Ca et Ba:Ca dans les écailles étaient hautement corrélés aux concentrations dans les otolithes des poissons soumis au même traitement. Globalement, les signatures de Sr:Ca, Cd:Ca et Ba:Ca dans les écailles semblent représentatives du milieu ambiant, et peuvent donc être utiles pour quantifier les caractéristiques du cycle biologique de chaque poisson.

[Traduit par la Rédaction]

Introduction

The elemental composition of fish bony structures, such as fin rays, scales, and otoliths, may be useful natural markers of population structure and habitat associations. Such elemental tags are available on every fish throughout their lives, as each fish is affected independently by its environment. Therefore, elemental analysis of bony structures can play a vital role in stock discrimination and the estimation of life-history parameters. Geochemical signatures in otoliths have been used to determine nursery-area residency, gain information on parentage, and estimate larval-dispersal path-

ways and adult-migration routes (reviewed by Campana 1999). Analyses of trace elements in scales have been used to identify poached fish (Belanger et al. 1987), understand stock structure (Pender and Griffin 1996), determine marine residency (Bagenal et al. 1973), and identify nursery areas (Coutant and Chen 1993). Importantly, scales offer a non-lethal approach that is not an option with otoliths and, thus, may be particularly useful when applied to rare or endangered species.

Before scales can be used to reconstruct environmental histories of individual fish, it is necessary to validate the assumption that trace elements in scales are deposited in proportion to dissolved concentrations in the ambient environment. To date, only qualitative observation and experimentation have been performed to describe the relationship. Yamada et al. (1979) and Yamada and Mulligan (1982, 1987) demonstrated that increasing Sr concentration in the environment marks the scale. Doubling or tripling Sr concentrations in the water has been shown to produce a corresponding two- and three-fold increase in scale Sr levels (Snyder et al. 1992). van Coillie and Rousseau (1974) demonstrated that, in polluted waters, excess metal ions from

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B.K. Wells,¹ G.E. Bath, S.R. Thorrold, and C.M. Jones.
Department of Biological Sciences, Old Dominion University,
Norfolk, VA 23529, U.S.A.

¹Author to whom all correspondence should be addressed at
Maryland Fishery Resource Office, U.S. Fish and Wildlife
Service, 177 Admiral Cochrane Drive, Annapolis,
MD 21401, U.S.A. e-mail: Brian_Wells@fws.gov

water (Cr, Mn, Co, Ni, Sr, Ag, Cd, Cs, Hg, and Pb) replaced Ca in scale apatite. Ennever and Beames (1993) exposed juvenile salmonids to elevated levels of lanthanum, cerium, and samarium by adding each of the three elements to the water in which the fish were reared. Resulting levels of the elements in scales were higher than in either otoliths or vertebrae, suggesting that scales may better reflect trace-element concentrations in the environment.

Accurate descriptions of the relationship between scale and water chemistry, as modified by physical variables such as temperature, requires detailed knowledge of environmental exposures of individual fish. This is most easily achieved under controlled laboratory conditions. For instance, laboratory experiments have documented the effects of temperature (e.g., Radtke 1989; Townsend et al. 1992), salinity (Fowler et al. 1995a, 1995b), diet (Gallaher and Kingsford 1996), and environmental element concentration (Farrell and Campana 1996; Bath et al. 2000) on otolith composition. Such research for scales, however, is limited (Gauldie et al. 1990, 1991; Mugiya et al. 1991).

In this study, we examined the relationship between Sr, Cd, and Ba levels in seawater and the resultant incorporation of these elements in the scales of juvenile spot, *Leiostomus xanthurus*. Sr and Ba are commonly assayed in otoliths and scales as they can be used to indicate movement in and out of an estuary as well as to discriminate between different estuaries (Wells et al. 2000). In oceans, Cd follows a nutrient-type distribution, with much greater concentrations in deep water than in shallow water, so it can be used to indicate exposure to upwelling waters. Further, anthropogenic inputs in rivers and estuaries can lead to very high concentrations of Cd. By maintaining the fish in the laboratory, we were able to manipulate and quantify exposures to both water temperatures and dissolved concentrations of the elements. We were also able to compare and contrast scale chemistry with that of otoliths from the same fish (results reported elsewhere (Bath et al. 2000)).

Methods

Experimental conditions

Spot were spawned on 22 November 1997 at the National Marine Fisheries Service Laboratory (Beaufort, N.C., U.S.A.) in a flow-through natural-seawater system (30‰ salinity), assuring that larvae were from the same brood stock and of known age. Thirty-five days after hatching, the larvae were transported to a rearing laboratory and transferred to 24 acid-washed high-density polyethylene tanks (20-L capacity) in a clean facility equipped with a positive flow air filter (0.2 µm) system. The fish were acclimated to artificial seawater (20‰, "Instant Ocean") for 4 days and were raised under experimental conditions for 42 days. Enriched *Artemia* were fed to the fish for the first 2 weeks of the experiment and, thereafter, fish were fed an artificial diet. Mortality over the course of the experiment was low, with an average of seven fish used from each tank for scale analyses (Table 1). Any fish that died before the designated end of the experiment were not used in any analyses.

Triplicate experimental tanks within each temperature treatment (20 and 25°C) were randomly assigned one of four levels of Sr:Ca, corresponding to baseline and 1.2×, 1.4×, and 1.8× baseline level, and four levels of Ba:Ca, corresponding to baseline and 3×, 6×, and 10× baseline level. Mean concentration levels for Cd:Ca over the experiment were baseline and 1.5×, 2×, and 3.5× baseline level.

After the first 3 weeks, we doubled the spiked levels of Cd:Ca, owing to difficulties in maintaining dissolved concentrations in the tanks. Baseline concentrations for each element were determined by inductively coupled plasma mass spectrometry (ICP-MS) assays of the experimental artificial seawater, at the correct salinity, before the start of the experiment (Table 1). The spiked waters were prepared by adding appropriate amounts of standard solutions (SPEX) of SrCl₂, CdCl₂, and BaCl₂ to each of the tanks.

Water chemistry

To maintain water quality and spike levels in the tanks, water was changed at 50% volume daily. Temperature, salinity, and pH were measured throughout the experiment and water samples were analyzed from each tank at weekly intervals, including the first and last days of the experiment. Water samples were filtered through a 0.22-µm cellulose nitrate membrane filter, acidified with trace metal grade 12 N HCl to pH 2, and stored frozen and acidified for subsequent analysis (Bath et al. 2000). In all data analyses, the average of each tank's 6-weekly values was used (Table 1).

We encountered two difficulties using artificial seawater in the experiment. First, Sr:Ca ratios in the "Instant Ocean" salts that we used were slightly higher (12 mmol·mol⁻¹) than those of typical seawater (8.5–9 mmol·mol⁻¹). Because dilution would only lower absolute Sr levels and would not change Sr:Ca ratios in the water, we could do little to lower this value. Therefore, the highest Sr:Ca values in this experiment were 2.5× those of normal seawater. We did not face this problem with baseline and spiked Ba:Ca levels, which spanned a range (23–230 µmol·mol⁻¹) that would commonly be encountered by estuarine-dependent fish along the east coast of the United States (Coffey et al. 1997). Cd:Ca levels were higher than those from pristine environments but were possible for polluted waters. Second, our decision to increase spiked levels of Cd halfway through the experiment, to achieve better separation among treatments, meant that within-tank variances of Cd:Ca were considerably higher than those for either Sr:Ca or Ba:Ca. The increased variance in water chemistry was also, in all probability, manifested in concomitant within-tank variability in scale Cd:Ca ratios.

Scale collection

One hundred and sixty-seven fish were used for scale analysis. Scales were removed from the entire fish with acid-washed glass probes and were washed twice with Milli-Q water. The scales were stored dry in acid-washed low-density polyethylene vials prior to the ICP-MS analyses. To ensure sufficient scale material for trace-element analysis, scales from individual fish within a tank were pooled into three randomly assigned groups and analyzed as a pooled sample. This practice maintained much of the within-tank variance, while still yielding enough scale material to obtain adequate results. Pooled scale sample weights ranged from 0.4 to 6.5 mg, with a mean weight of 1.6 mg.

Scale analysis

Elemental analyses of scales were conducted using ICP-MS; for quantification, both isotope dilution (Sr, Cd, and Ba) and internal standardization (Ca) were used. Scales were dissolved in high-purity nitric acid (Seastar Chemicals Inc.) that contained enriched isotopes (⁸⁷Sr, ¹¹²Cd, and ¹³⁷Ba) and an internal standard (⁴⁵Sc). These isotopes, along with ⁴⁸Ca, ⁸⁸Sr, ¹¹⁴Cd, and ¹³⁸Ba, were then monitored in the samples for quantification purposes. We confirmed that all isotopes were free of isobaric interferences, using high resolution ICP-MS (Thorrold and Shuttleworth 2000). Blank values based on analyses of high-purity nitric acid were less than 100 µg·g⁻¹ for Ca, less than 1 µg·g⁻¹ for Sr, less than 0.1 µg·g⁻¹ for Cd, and less than 0.05 µg·g⁻¹ for Ba. ICP-MS operating conditions are shown in Table 2. Scale samples were blocked by tank, so that a sample from each of the 24 tanks was assayed in turn. The order

Table 1. Summary of average temperature (T); pH; dissolved Sr:Ca ($\text{mmol}\cdot\text{mol}^{-1}$), Cd:Ca ($\mu\text{mol}\cdot\text{mol}^{-1}$), and Ba:Ca ($\mu\text{mol}\cdot\text{mol}^{-1}$); standard length (SL); the number of fish used from each tank; and the number of pooled samples analyzed from each tank.

| Tank | T ($^{\circ}\text{C}$) | pH | Sr:Ca | Cd:Ca | Ba:Ca | SL | No. fish | No. pooled samples |
|------|----------------------------|------|-------|-------|--------|-------|----------|--------------------|
| 1 | 20.8 | 8.00 | 15.21 | 19.53 | 151.16 | 24.59 | 9 | 3 |
| 2 | 25.2 | 8.05 | 17.40 | 22.41 | 71.41 | 20.21 | 6 | 3 |
| 3 | 25.2 | 8.00 | 16.03 | 14.84 | 25.48 | 27.46 | 11 | 3 |
| 4 | 20.3 | 7.97 | 15.17 | 26.37 | 23.90 | 23.05 | 5 | 3 |
| 5 | 20.6 | 7.96 | 22.36 | 26.97 | 138.83 | 24.06 | 4 | 3 |
| 6 | 20.4 | 8.02 | 17.68 | 18.16 | 72.30 | 25.27 | 1 | 1 |
| 7 | 24.8 | 8.01 | 22.74 | 29.79 | 148.14 | 23.84 | 8 | 3 |
| 8 | 25.2 | 7.98 | 17.88 | 30.33 | 20.85 | 24.02 | 11 | 3 |
| 9 | 25.1 | 8.00 | 22.47 | 12.18 | 22.93 | 22.52 | 9 | 3 |
| 10 | 20.3 | 7.95 | 12.73 | 22.44 | 155.85 | 24.39 | 9 | 3 |
| 11 | 20.4 | 7.91 | 12.54 | 20.01 | 228.09 | 25.83 | 10 | 3 |
| 12 | 25.4 | 8.04 | 22.50 | 49.75 | 70.21 | 23.00 | 10 | 3 |
| 13 | 20.3 | 7.97 | 17.79 | 48.49 | 22.98 | 24.42 | 5 | 3 |
| 14 | 20.3 | 7.94 | 22.55 | 53.65 | 215.56 | 24.46 | 9 | 3 |
| 15 | 25.1 | 8.04 | 12.75 | 29.31 | 211.75 | 21.89 | 5 | 2 |
| 16 | 25.0 | 8.03 | 15.23 | 24.78 | 144.87 | 22.88 | 5 | 3 |
| 17 | 24.7 | 8.02 | 13.03 | 46.67 | 74.27 | — | — | — |
| 18 | 25.5 | 7.98 | 18.29 | 11.49 | 231.45 | 24.41 | 9 | 3 |
| 19 | 20.3 | 8.00 | 17.81 | 12.01 | 75.09 | 25.71 | 7 | 3 |
| 20 | 24.9 | 8.00 | 13.39 | 49.66 | 142.93 | 23.61 | 5 | 3 |
| 21 | 19.7 | 7.98 | 15.00 | 50.14 | 70.96 | 24.78 | 7 | 3 |
| 22 | 20.4 | 8.01 | 12.60 | 32.56 | 222.20 | 23.15 | 6 | 3 |
| 23 | 24.5 | 8.01 | 15.02 | 22.73 | 24.88 | 24.17 | 8 | 3 |
| 24 | 20.0 | 7.98 | 22.80 | 14.39 | 23.19 | 26.56 | 8 | 3 |

Table 2. Inductively coupled plasma mass spectrometry (ICP-MS) system operating conditions.

| (A) General | | |
|----------------------------------|-------------------------------------|--------------------------|
| Instrument | ICP-MS | |
| Make | Turner Scientific (Finnegan/MAT) | |
| Model | Sola | |
| R. f. power | 1500 W | |
| Sample gas flow rate | 0.8 mL·min ⁻¹ | |
| Nebulizer gas flow rate | 0.9 L·min ⁻¹ | |
| Auxiliary gas flow rate | 1.2 L·min ⁻¹ | |
| Coolant flow rate | 15 L·min ⁻¹ | |
| (B) Specific | | |
| | For the Ca and Sr run | For the Cd and Ba run |
| Detector | Multiplier | Faraday |
| Channels per atomic mass unit | 8 | 8 |
| Passes per scan | 16 | 4 |
| Dwell time | 8 ms | 64 ms |
| Number of scans | 1 | 5 |
| Resolution | 30 | 30 |

in which tanks were sampled within each of the three blocks was randomized. Elemental concentrations were standardized to Ca, because the elements examined are likely to substitute for Ca in the bone matrix (van Coillie and Rousseau 1974; Campana 1999). The incorporation of Sr, Cd, and Ba ions into the hydroxyapatite will,

therefore, be dependent upon the ratios of these elements to Ca in the depositional fluid and not upon the absolute concentration of the elements themselves.

Data analysis

Least-squares regression was used to evaluate the significance of our treatments and the assimilation relationships. Elemental-analysis results were averaged for each tank and used in the statistical analyses. The otolith chemistry was determined for each fish used in the scale analysis and is reported elsewhere (Bath et al. 2000). We compared the chemistry of scales and otoliths from juvenile spot using Pearson's correlation analysis, after averaging both otolith and scale data across each of the 24 tanks. Data from one tank (temperature = 25°C , Sr = $1\times$, Cd = $3.5\times$, and Ba = $3\times$) were removed, owing to measurement error or sample contamination, reducing the number of replicates for this treatment to two.

Variances in mean concentrations in water and scales among the four Sr:Ca and Cd:Ca treatments were equal and values were normally distributed. However, variances in the Ba:Ca treatments increased with increasing spike levels, which also appeared to be reflected in scale chemistry. Unfortunately natural log transformations of the data did not result in equal variances nor did they improve the distribution of residuals. Given the difficulties generally associated with transformations before regression analyses (Underwood 1997), we have presented both linear and curvilinear models for the Ba:Ca data.

Results

Juvenile spot collected from the experiment had standard lengths (SL) that ranged from 18.0 to 32.6 mm, with an overall mean of 24.2 mm. SLs were similar between the

temperature groups (t test, $T = 2.03$, $df = 21$, $p = 0.055$; Table 1). While these values were on the threshold of being statistically significant, mean lengths for the 20°C ($SL = 24.7 \pm 0.3$ SE) and 25°C ($SL = 23.5 \pm 0.5$ SE) treatments only differed by 1 mm (or ~5%), suggesting that temperature had little measurable effect on fish growth. Growth-rate differences did not, therefore, contribute significantly to variability in scale chemistry between temperature treatments.

Water chemistry

Results from a fixed factorial analysis of variance (ANOVA) showed that all three element:Ca ratios increased significantly with spiking levels (ANOVA, $F_{[3,15]} = 274.5$ – 1149.4 , $p < 0.0001$; Fig. 1). Also, element:Ca ratios were not influenced by temperature (ANOVA, $F_{[1,15]} = 0.1$ – 2.4 , $p > 0.05$).

Trace-element incorporation

All three element:Ca concentrations in the scales were significantly correlated with ambient levels in the tanks (Fig. 2). Results from analysis of covariance (ANCOVA) showed that temperature had no influence on the incorporation of Sr, Cd, or Ba (ANCOVA, $F_{[1,20]} = 0.1$ – 0.4 , $p > 0.05$) and, therefore, was dropped as a treatment effect in all subsequent statistical analyses.

Sr:Ca concentrations in scales ($[Sr:Ca]_{scale}$) were significantly correlated with Sr:Ca concentrations in the tanks ($[Sr:Ca]_{water}$) and were described by the following least-squares regression equation ($r^2 = 0.81$, $p < 0.0001$):

$$[Sr:Ca]_{scale} = (0.16 \pm 0.02 \text{ SE})[Sr:Ca]_{water} + (0.06 \pm 0.29 \text{ SE})$$

The intercept was not significantly different from zero, and constraining the line through the origin also resulted in a slope of 0.16.

Despite higher variation within individual tanks, Cd:Ca ratios in scales were also significantly correlated with Cd:Ca levels in the ambient tank waters, albeit with a relatively low coefficient of variation. The least-squares linear regression equation for these data ($r^2 = 0.18$, $p < 0.05$) was given by

$$[Cd:Ca]_{scale} = (0.44 \pm 0.20 \text{ SE})[Cd:Ca]_{water} + (18 \pm 6 \text{ SE})$$

The intercept of the line was significantly greater than zero, owing, at least in part, to increased variability at low Cd:Ca values in both ambient water and scales.

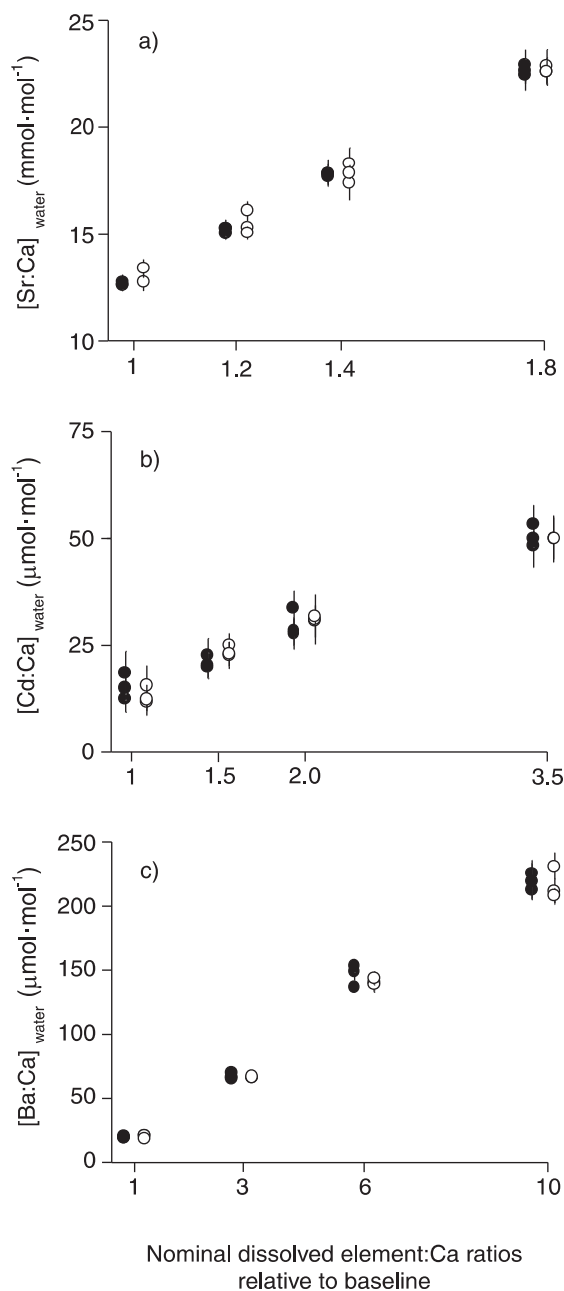
Ba incorporation in scales was highly correlated with ambient Ba levels in the tanks. Least-squares regression described a linear relationship between $[Ba:Ca]_{water}$ and $[Ba:Ca]_{scale}$ ($r^2 = 0.91$, $p < 0.0001$) and was given by

$$[Ba:Ca]_{scale} = (0.20 \pm 0.01 \text{ SE})[Ba:Ca]_{water} + (11 \pm 2 \text{ SE})$$

As with the Cd data, the intercept of the regression line was significantly greater than zero. A curvilinear function also fit ($r^2 = 0.95$, $p < 0.0001$), and was given by

$$\log_e[Ba:Ca]_{scale} = (0.61 \pm 0.03 \text{ SE}) \log_e[Ba:Ca]_{water} + \log_e(0.69 \pm 0.14 \text{ SE})$$

Fig. 1. The mean element:Ca concentration in the water from each tank plotted against the treatment concentration groups (± 1 SE) at 20°C (●) and 25°C (○) for Sr:Ca (a), Cd:Ca (b), and Ba:Ca (c). The x-axis shows the four different concentration treatments represented as relative multiples of the baseline concentrations.

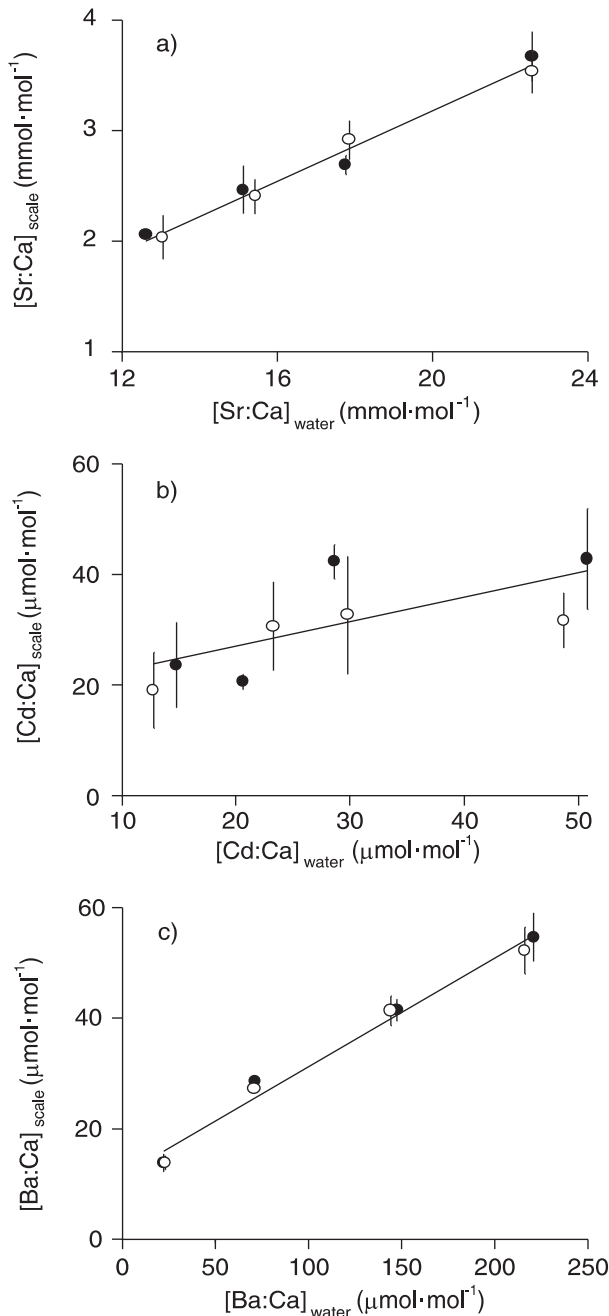


The fit of the curvilinear model ($r^2 = 0.95$) was only slightly better than the linear model ($r^2 = 0.91$) and did not improve the distributions of the model residuals. Therefore, for reasons of parsimony, only the linear function is presented graphically (Fig. 2).

Comparison of scale and otolith chemistry

Finally, we correlated Sr:Ca and Ba:Ca ratios in scales with the same ratios quantified in otoliths from the same fish, after first averaging data within tanks. The Cd:Ca con-

Fig. 2. Mean element:Ca concentrations in the scales from the eight treatment groups plotted against actual element:Ca concentrations in the water (± 1 SE) at 20°C (●) and 25°C (○) for Sr:Ca (a), Cd:Ca (b), and Ba:Ca (c). Lines represent the least-squares regressions.



concentrations in many otoliths were below detection limits and therefore could not be compared with scale data. Both Sr:Ca and Ba:Ca ratios from scales were highly correlated with values in otoliths (Fig. 3), with correlation coefficients of 0.91 and 0.94, respectively. Sr:Ca values were of similar magnitude in scales and otoliths, with otoliths having only slightly greater concentrations. In contrast, Ba:Ca ratios were approximately sixfold higher in scales than in otoliths. Furthermore, the Ba:Ca relationship between scales and otoliths was somewhat curvilinear. This indicated that the up-

take of Ba:Ca in scales may be nonlinear, especially for the higher-temperature treatment.

Discussion

We have shown that Sr:Ca, Cd:Ca, and Ba:Ca concentrations in spot scales reflect differences in elemental concentrations of the ambient environment. This, in turn, indicates that water chemistry is a major determinant of at least a subset of the elemental composition of scales. It may, therefore, be possible to reconstruct past environmental histories and, in particular, ambient dissolved Sr and Ba concentrations, of individual fish, based on geochemical analyses of scales. For instance, movement of fish from marine (high Sr, low Ba) to estuarine (low Sr, high Ba) waters should be accurately recorded by scale chemistries (e.g., Coutant and Chen 1993). Similarly, these data strengthen our conclusion that geographic differences in the scale geochemistry of juvenile weakfish (*Cynoscion regalis*) are a function of exposure to waters with different chemical characteristics (Wells et al. 2000).

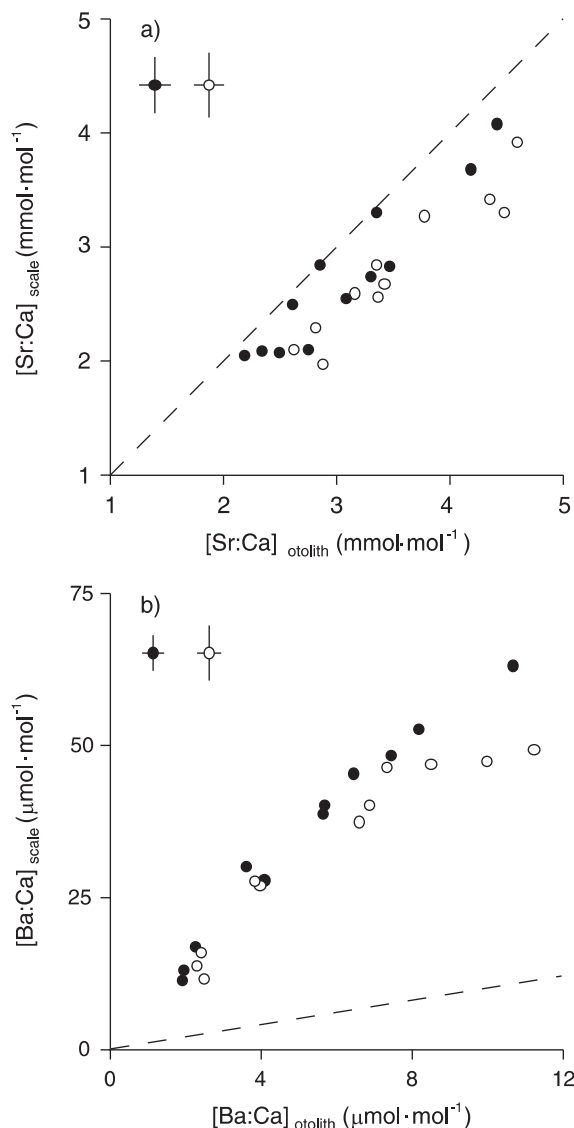
Temperature was not important in determining Sr:Ca, Cd:Ca, or Ba:Ca ratios in scales. While considerable debate continues over the influence of temperature on otolith chemistry (Campana 1999), there has been little discussion of the possible influences of temperature on scale chemistry. Bath et al. (2000) found that, unlike scales, Sr:Ca ratios in otoliths from the same fish used in the present study were related to temperature. This dichotomy may help to determine where the temperature effect in otolith Sr:Ca ratios is generated. Sr from ambient water that is subsequently deposited in scales and otoliths is transported to depositional sites by blood plasma (Takagi et al. 1989; Kalish 1991). Therefore, any temperature-dependent fractionation between Sr and Ca cannot be occurring at the interface between either the branchial or intestinal membrane and the ambient water, or within the blood plasma. Fractionation at either of these locations would have resulted in a significant temperature effect on scale Sr:Ca ratios in our experiment, because scales receive ions directly from the blood plasma (Takagi et al. 1989). It follows that temperature affects Sr:Ca ratios in otoliths either by influencing the transfer of Sr across the endolymphatic membrane or by determining the rate at which Sr ions substitute for Ca ions in the aragonite lattice. Nonetheless, reconstruction of Sr concentrations in waters based on scales rather than otoliths has an advantage, because variations in Sr:Ca ratios can be attributed unambiguously to differences in water chemistry and not to temperature.

The slope of the relationship between $[\text{Sr:Ca}]_{\text{scale}}$ and $[\text{Sr:Ca}]_{\text{water}}$ provides an estimate of the partition coefficient (D_{Sr}) between ambient water and the scale, where

$$[\text{Sr:Ca}]_{\text{scale}} = D_{\text{Sr}}[\text{Sr:Ca}]_{\text{water}}$$

with the intercept forced through the origin. We found a value of 0.16, which is similar to the value for otoliths from the same fish ($D_{\text{Sr}} \sim 0.19$; Bath et al. 2000). It may be wrong to conclude, however, that Sr has a comparable affinity for scale hydroxyapatite and otolith aragonite based on these data. Scale apatite incorporates elements directly from the blood plasma, while otoliths incorporate elements from the endolymphatic fluid. Kalish (1991) noted that Sr:Ca concen-

Fig. 3. A comparison of the average element:Ca concentration in the scales from each tank with the average element:Ca concentration in the otoliths from the same tank at 20°C (●) and 25°C (○) for Sr:Ca (a) and Ba:Ca (b). The broken line indicates a 1:1 relationship between scales and otoliths. The average SEs across all tanks within each temperature group are shown for otoliths and scales.



trations are, in fact, higher in blood plasma than in the endolymphatic fluid. If this were also the case with juvenile spot, it follows that Sr substitutes more easily for Ca in otolith aragonite than in scale apatite. Given results from a comparison of Sr:Ca values in scales and otoliths from field-caught weakfish, which were strikingly similar to comparisons made here, Wells et al. (2000) came to the same conclusion.

A rough estimate of the Ba:Ca partition coefficient for scale apatite, based on the slope of the relationship between $[\text{Ba:Ca}]_{\text{scale}}$ and $[\text{Ba:Ca}]_{\text{water}}$, was 0.20. Surprisingly, this value is much larger than the otolith partition coefficient (~ 0.06 ; Bath et al. 2000). The absence of any data on Ba levels in the blood plasma or endolymphatic fluid of fish meant that we could not determine if the increased affinity for Ba in scales compared with that for otoliths was occurring during

uptake or transport in the blood plasma, or at the depositional surface. The relationship between $[\text{Ba:Ca}]_{\text{scale}}$ and $[\text{Ba:Ca}]_{\text{water}}$ was also noteworthy, because the intercept of the relationship did not pass through the origin. This implies that Ba will be present in the scale even when there is no Ba in the water. Although such a result would obviously seem illogical in an inorganic system, fish scales contain significant quantities of protein, along with hydroxyapatite, that may also be a source of Ba. The organic component of scales may have contributed the excess Ba and we suspect that the regression line would have gone through the origin if the protein material had been removed before chemical assay. The alternative hypothesis is that the relationship between $[\text{Ba:Ca}]_{\text{scale}}$ and $[\text{Ba:Ca}]_{\text{water}}$ is curvilinear rather than linear, since a curvilinear relationship would, by definition, pass through the origin. Although we presented the results of a linear regression for easier comparison with earlier studies, there was little difference between this relationship and a curvilinear least-squares regression. Interestingly, the relationship between otolith and scale Ba:Ca ratios appeared to be approaching an asymptote, especially for the 25°C tanks. However, a comparison of Ba:Ca values in scales and otoliths from weakfish caught in varied environments along the Atlantic coast of the United States showed no indication of being asymptotic through the data up to scale values of $300 \mu\text{mol}\cdot\text{mol}^{-1}$ (Wells et al. 2000).

While the relationship between $[\text{Cd:Ca}]_{\text{scale}}$ and $[\text{Cd:Ca}]_{\text{water}}$ was not very precise, Cd:Ca ratios were similar in scales and water. This contrasts with otoliths, in which it is probably safe to assume that the partition coefficient is somewhat less than 0.0001 (Campana 1999). It is possible that the endolymphatic fluid does not accumulate Cd ions to the same extent as blood plasma (Campana (1999) reported that Cd concentration in blood plasma may be $3000\times$ that of the ambient environment). However, this would require that the endolymphatic membrane be able to actively discriminate against Cd ions when the branchial and intestinal membranes apparently cannot. Alternatively, Cd may substitute more easily for Ca in hydroxyapatite than in aragonite. Indeed, Cd:Ca in scales can be 35–55% that of Ba:Ca and 22–30% that of Sr:Ca (van Coillie and Rousseau 1974). The organic component of the scales may also have contributed to the high total Cd levels. This may, in turn, explain the variability in the relationship between $[\text{Cd:Ca}]_{\text{scale}}$ and $[\text{Cd:Ca}]_{\text{water}}$ since it is unlikely that the concentration of organically bound Cd would be a function of water chemistry.

The results from this work are limited to nonessential elements that are able to substitute for Ca in the hydroxyapatite matrix of scales. Our results will, in all likelihood, not apply to physiologically controlled elements (e.g., Na, K, Mg, Cl, P, Cu, and S; Campana 1999), as their levels in blood plasma are more a function of active regulation by the fish than of environmental concentration. Interestingly, Mugiya et al. (1991) found that of 10 elements examined (Al, Cd, Ba, Mn, Fe, Ni, Cu, Zn, Pb, and Sr) in scales and otoliths, only Sr and Ba were deposited in proportion to availability in the environment for both hard parts.

If scale geochemistry is to be used as a proxy for past environmental conditions, material in scales must remain unmodified after deposition. Although otoliths are generally considered to be metabolically inert after crystallization, few

studies have examined the stability of geochemical signatures in scales. There is evidence that scales may cease to grow, or even be resorbed, during times of physiological stress (Bilton and Robins 1971; Bilton 1975). However, Yamada and Mulligan (1982) reported that induced Sr tags in scales remained constant for at least 3 years and suggested that, once Sr was incorporated into the scale, it became metabolically inert. Studies of other hydroxyapatite structures have suggested that such metabolic reworking may also be rare, at least for those elements commonly assayed in scales (Wells et al. 2000). For instance, Rosenthal (1963) found that Sr in fin rays had a biological half-life of at least 2.5 years. More recently, Veinott and Evans (1999) noted that minor- and trace-element measurements (Mg, Mn, Sr, and Ba) of fin rays made 2 years apart remained highly correlated (r values for all elements were ≥ 0.88). Thus, while more research is clearly needed on the stability of scale chemistry, it appears that the half-lives of at least some elements in scales may be sufficiently long to be useful as environmental tracers.

The use of scales to identify elemental signatures offers certain advantages and disadvantages over using elemental signatures in otoliths. Scale analysis offers a nonlethal alternative to otoliths, and scales are generally easy to collect, archive, and prepare for analysis. However, scales are not present on fish during most of the larval period and, therefore, are not suited to questions of larval dispersal (e.g., Swearer et al. 1999). Scales are typically more common in sediment cores and institutional collections than otoliths. Further, time periods represented by circuli on the scale can be assayed without grinding, because each circulus is exposed throughout a fish's life. This, in turn, eliminates the possibility of contamination from the grinding material. Although elemental concentrations were generally higher in scales than in otoliths, we found that elemental concentrations in otoliths were more tightly correlated with ambient water chemistry than those in scales (Bath et al. 2000). Finally, the observations that otoliths are metabolically inert, even under extreme stress (Campana and Neilson 1985), suggests that otoliths will remain the structure of choice for elemental and isotopic analyses when available. However, providing that elemental signatures remain sufficiently stable throughout a fish's life, scales may offer a nonlethal alternative for determining retrospective information of environmental conditions experienced by an individual fish. This information could then be used as natural tags for population structure, habitat use, and migration pathways, in a similar way that such information is currently used in otolith studies.

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